Egyptian Journal of Chest Diseases and Tuberculosis (2014) 63, 907–911

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Egyptian Journal of Chest Diseases and Tuberculosis

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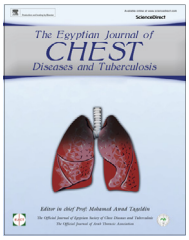

ORIGINAL ARTICLE

Assessment of the histopathological prototypes of the pleural fluid and granulomatous tissue reaction associated with pleural tuberculosis

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Received 20 June 2014; accepted 10 July 2014
Available online 5 August 2014

KEYWORD

Free-encysted-tuberculosis-granulomatous

Abstract *Purpose:* The aim of this study is to differentiate between the histopathological features of pleural tuberculosis both free and encysted forms of pleural effusion as regards pleural fluid cytology and pleural tissue granulomatous tissue reaction and assess the effects of combined microbial infection on histopathological and clinical residuals.

Patients and methods: One hundred and ninety patients were confirmed to be tuberculous by pleural tissue biopsy. Pleural fluid cytopathology and TB granulomatous tissue biopsy samples were studied.

Results: Within histopathological features in TB pleural effusion is typical granulomatous TB reaction that was prevalent in both free and encysted forms of pleural effusion with high percentage (97% and 87.5% respectively). On the other hand, lymphocytic exudative smear was predominant in the free TB effusion group than both inflammatory and hemorrhagic ones (74.7% versus 23.5% and 1.8% respectively) while the encysted effusion group presented higher predominance to inflammatory exudative smear than other types (54.2% versus 41.7% and 4.2% respectively). Moderate cellularity was higher in percentage in both free and encysted groups 77.7% and 62.5% respectively. Also, langerhans cells prevailed in both effusion types. Lastly, non TB culture was present in all cases of encysted pleural effusions but accounted only for 3% of free effusion cases.

Conclusion: Differentiation between free and encysted forms of pleural tuberculous effusion as regards effusion cytopathological criteria and pleural tissue histopathological patterns represent an important support in understanding the pathogenesis of pleural tuberculous tissue reactions and other positive cultural diagnosis can aggravate TB granulomatous reactions.

Introduction

Histolopathological diagnosis of tuberculosis (TB) has long been an important issue in anatomical pathology. On the other hand, pleural TB comprises 10–15% of infections with MTB.

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Peer review under responsibility of The Egyptian Society of Chest Diseases and Tuberculosis.

http://dx.doi.org/10.1016/j.ejcdt.2014.07.011

A definite diagnosis of tuberculous pleurisy is achieved when *Mycobacterium tuberculosis* is demonstrated in sputum or pleural specimens, or when caseous granulomas are found in pleural biopsies [1,2]. A tuberculous effusion is usually serous, may be serosanguineous, but is almost never frankly bloody. Examination of pleural fluid is diagnostic of tuberculosis only if mycobacteria are demonstrated in an exudative smear or culture. Unfortunately this is an uncommon occurrence as mycobacteria are demonstrable on exudative smear in less than 10% of patients and on culture in only 25% of patients. Decisions regarding treatment are usually made without confirmatory stains and well before the culture results are available. Certain features of tuberculous pleural fluid are helpful in either supporting or discounting the diagnosis of tuberculosis. Typically, more than 50% of all white blood cells in a tuberculous pleural effusion are mature lymphocytes, and a differential count that reveals more than 80% mature lymphocytes strongly suggests either tuberculosis or malignancy. Eosinophil count rarely exceeds 10% in tuberculous pleural fluid. Mesothelial cells are rare; indeed, more than 5% mesothelial cells in the differential count argue strongly against a tuberculous etiology. Biopsy of the pleura demonstrates granuloma in approximately 80% of patients. Pleural tuberculosis is the only nonneoplastic pleural exudate readily diagnosed by a pleural biopsy. Culture of the pleural biopsy is helpful as well since *M. tuberculosis* can be isolated from over 85% of biopsies. Although other diseases, including fungal infection, sarcoidosis, and rheumatoid arthritis may produce granulomatous pleuritis, more than 95% of patients with demonstrable pleural granuloma have tuberculosis [3].

Considering the limitations in sensitivity and specificity of Ziehl–Neelsen staining [4], mycobacterial evaluation, mycobacterial culture, molecular, serological techniques [5–7], and histomorphological analysis appears to be the only feasible technique for the field diagnosis of TB in some patients [8,9]. Granulomatous reactions, and in some cases, nongranulomatous reactions such as the presence of foamy macrophages [10] or mycobacterial spindle cell pseudotumor [11] in some types of mycobacterial infections are indicative of TB only if the presence of TB bacilli has been confirmed in the tissue. Recently, it has been stated that PCR results are acceptable only if the presence of TB bacilli can be confirmed in the tissue [12].

Subjects and methods

A total of 190 patients who underwent simultaneous pleural fluid cytology biopsies and tissue cultures with positive tissue cultures for MTB during the period between 2010 and 2013 were selected from the Chest Department Mansoura University Hospitals. These cases with tuberculous pleural effusion were biopsied using pleural Abrams needle over the side and site of the maximal fluid retrieval. Cases were prepared in semi sitting position with sterilization of the area identified for biopsy taking anesthetized by lidocaine 5%, simple puncture using three way cannula 22 gauge for aspiration of pleural fluid followed by small skin snipping using 11 scalpel, trocar of pleural Abrams needle was introduced into parietal pleural snips where were obtained in at least 6 directions aiming to gain sufficient amount of parietal pleural tissue. Skin was sutured using 2 or 3 zero of silk ampoules followed by gauze packing. Immediate thoracic ultrasound and Chest X-ray were done to secure against any complication 1 h and 6 h later. Cases with small number of atypical granulomatous pleural lesions or tissue volume were excluded from the study. Eventually, 190 tissue samples of 190 TB patients with adequate tissue and number of granuloma were chosen for different staining techniques. Characteristics and type of samples were retrieved from the pathology reports. Paraffin-embedded blocks were stained using Ziehl–Neelsen stain. Ziehl–Neelsen staining was performed according to the standard protocol. In summary, tissue specimens were deparaffinized and rinsed with consecutive dilutions of alcohol [96–70% ethanol]. After heat fixation, specimens were washed with carbol fuchsin for 4 min and incubated with HCl. Counterstaining was done using Brilliant Green dye for 20 s. After rinsing, samples were allowed to dry at room temperature.

Statistical analysis

Data were analyzed using SPSS (Statistical Package for Social Sciences) version 16. Qualitative data were presented as numbers and percentages. Quantitative data were presented for normality by the Kolmogorov–Smirnov test. Normally distributed data were presented as mean and standard deviation. Comparison between groups was done using Chi-square test. Student's *t*-test was used to compare between two groups. P value < 0.05 was considered significant.

Results

As shown in Table 1 our study was conducted on 190 patients (135 males and 55 females). Mean age was 37.54 ± 15.25 years (range 14–76 y) in total cases studied. The mean age of patients in free TB effusion group was 37.259 ± 15.691 years and 38.166 ± 16.21 years in encysted TB effusion group with highly significant statistical difference as regards age ($P = 0.00$).

Patients in the free group, had higher percentage of males than females (72.3% and 27.7% respectively), the encysted group patients also had more males than females (62.5% and 37.5% respectively) with non-significant statistical difference as regards sex (P value > 0.005).

Table 2 shows histopathological features in TB pleural effusion that is classified into 5 prototypes. Granulomatous TB reaction subdivided into typical and nontypical caseating granuloma. Typical form was prevalent in both free and encysted pleural effusions with high percentage (97% and 87.5% respectively) with significant statistical difference between two groups. On the other hand, lymphocytic exudative smear was predominant in the free TB effusion group than both inflammatory and hemorrhagic ones (74.7% versus 23.5% and 1.8% respectively) while the encysted effusion group presented higher predominance to inflammatory exudative smear than other types (54.2% versus 41.7% and 4.2% respectively) with significant statistical difference between two groups. Moderate cellularity accounted for higher percentage in both free and encysted groups is 77.7% and 62.5% respectively without significant statistical difference between two groups. Also, langerhans cells prevailed in both effusion types without significant statistical difference between two groups. Lastly, non TB culture was present in all cases of

Table 1 Relation between age, sex and TB pleural effusion.

	Free TB pleural effusion group [n = 166]	Encysted TB pleural effusion group [n = 24]	P value
Age			
	Mean ± SD (37.259 ± 15.691)	Mean ± SD (38.166 ± 16.21)	0.000
Sex			
Male	120 (72.3%)	15 (62.5%)	0.323
Female	46 (27.7%)	9 (37.5%)	0.544
Total	37.54 ± 15.25 (range 14–76 y)		

Table 2 Histopathological features in TB pleural effusion.

Histopathological features	Free TB pleural effusion group (n = 166)	Encysted TB pleural effusion group (n = 24)	P value
1. Granulomatous reaction			
Typical caseation	161 (97%)	21 (87.5%)	0.031
Nontypical caseation	5 (3%)	3 (12.5%)	
2. Exudative smear predominant cell			
Hemorrhagic	3 (1.8%)	1 (4.2%)	0.004
Inflammatory	39 (23.5%)	13 (54.2%)	
Lymphocytic	124 (74.7%)	10 (41.7%)	
3. Degree of cellularity			
High	21 (12.7%)	3 (12.5%)	0.171
Moderate	129 (77.7%)	15 (62.5%)	
Scanty	16 (9.6%)	6 (25%)	
4. Special cell types			
Langerhans cell	95 (57.2%)	12 (50%)	0.398
Mesothelial cell	20 (12%)	1 (4.2%)	
Histiocytes	3 (1.8%)	0 (0%)	
Bilharzial	1 (0.6%)	0 (0%)	
Non	47 (28.3%)	11 (45.8%)	
5. Non TB culture			
Positive	5 (3%)	24 (100%)	0.000
Negative	161 (97%)	0 (0%)	

encysted pleural effusion but accounted only for 3% of free effusion cases with highly significant statistical difference between two groups (P = 0.00).

Fig. 1 illustrates that typical granulomatous caseation, lymphocytic exudative smear, moderate cellularity, presence of langerhans cells, and negative non TB culture showed higher percentage than encysted TB effusion on the contrary nontypical caseation, inflammatory smear, absence of special types of cells and positive non TB culture predominate in encysted TB effusion.

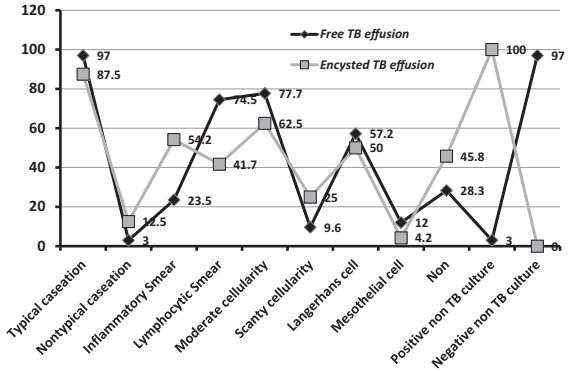

Figure 1 Histopathological features of TB effusion.

Discussion

In our study, histopathological features in TB pleural effusion are classified into 5 types, granulomatous tuberculous caseation reaction, exudative smear predominance, degree of cellularity, special types of cells and presence or absence of nontuberculous culture. Ziehl–Neelsen stain method for the diagnosis of TB is positive in all cases with confirmed TB infection. Detection of tuberculosis in tissue slides is still based on the histological pattern of the granuloma [13].

Granulomatous TB reaction in turn is subdivided into typical and nontypical caseating granuloma, typical form was prevalent in both free and encysted pleural effusion with high percentage (97% and 87.5% respectively) with significant statistical difference between two groups owing to the same causative organism and the principal offending pathogenesis. On the other hand, lymphocytic smear was predominant in the free TB effusion group than both inflammatory and hemorrhagic ones (74.7% versus 23.5% and 1.8% respectively)

while the encysted effusion group presented higher predominance to inflammatory exudative smear than other types (54.2% versus 41.7% and 4.2% respectively) with significant statistical difference between two groups particularly due to combined primary and secondary factors and different pathogenesis. Moderate cellularity accounted for higher percentage in both free and encysted groups (77.7% and 62.5% respectively) without significant statistical difference between two groups however, higher cellularity illustrated in both groups (12.5%) was correlated with the severity of infection. Also, langerhans cells prevailed in both effusion types without significant statistical difference between two groups. Lastly, non TB culture was present in all the cases of encysted pleural effusion but accounted only for 3% of free effusion cases with highly significant statistical difference between two groups ($P = 0.00$).

This issue has been well described by Velayati et al. [14]. A similar study [15] mentioned the presence of mycobacterium and intracellular mycobacterium material outside the granuloma and in macrophages and even lymphocytic fragments around the granuloma. Recent studies are also indicative of the active role of mesothelial cells in immunologic tissue reactions [16].

All the cases studied by Amitabha et al., had predominantly lymphocytic exudative pleural effusion and 89.5% of them had lymphocyte more than 75% in their pleural fluid. According to Light, [17] in tubercular pleural effusion, the pleural fluid lymphocyte is usually more than 50%. Occasional mesothelial cells were found in 28.9% of cases, and in no case, it was more than 3%. So, in pleural effusion, mesothelial cells of more than 5% strongly argue against a tubercular etiology [18,19]. The positive biopsy finding was defined as the presence of the caseating epithelioid cell granuloma with Langhan's type of foreign body giant cell. However, the absence of caseation did not rule out the diagnosis. Though similar type of findings could be found in some other conditions as well [e.g. fungal infection], but they are so rare in occurrence that in all practical cases, this finding is enough to be synonymous with the tubercular granuloma [17]. There have been multiple reports about following-up the cases with the pleural biopsy report of non-specific pleuritis. In the series by Onadeko, et al. it was reported that 54% of cases with chronic non-specific pleuritis were finally diagnosed as tubercular pleuritis [20]. In India also, there are various study reports regarding diagnostic yield of pleural biopsy. Maldhure et al. in the year of 1994 published a study report on comparative efficacy of pleural biopsy and increased pleural fluid ADA level in 83 cases of suspected tubercular pleural effusion. The pleural biopsy report was consistent with tuberculosis in 67.07% cases [21]. In a review of 14 papers from 1958 to 1985, encompassing a total of 2893 pleural biopsies, the diagnostic yield was 75% with tubercular pleurisy [22]. Three cases (7.8%) showed AFB on tissue biopsy which is much lower than 25.8% as detected by Valdes et al.

Conclusion

The most common way to make the diagnosis of tuberculous pleuritis over the past 50 years has been with a blind needle biopsy of the pleura. The demonstration of the granuloma in the parietal pleura suggests tuberculous pleuritis; caseous necrosis and AFB need not be demonstrated. Differentiation between free and encysted forms of pleural tuberculous effusion as regards effusion cytopathological criteria and pleural tissue histopathological patterns represents an important support in understanding the pathogenesis of pleural tuberculous tissue reactions and cultural diagnosis of other organisms can also add further load on pleural tissue by combined pathological reaction.

Conflict of Interest

There is no conflict of interests in this study.

References

[1] H.W. Berger, E. Mejia, Tuberculous pleurisy, Chest 63 (1973) 88–92.
[2] C.E. Bueno, G. Clemente, B.C. Castro, et al, Cytologic and bacteriologic analysis of fluid and pleural biopsy specimens with cope's needle, Arch. Intern. Med. 150 (1990) 1190–1194.
[3] L. Martin, Mayse: Nonmalignant pleural effusion, in: Alfred Fishman (Ed.), Fishman's Pulmonary Diseases and Disorders, fourth ed., McGraw-Hill Companies, Chap. 85(3) (1998) 1493–1494.
[4] A. Trusov, R. Bumgarner, R. Valijev, et al, Comparison of Lumin LED fluorescent attachment, fluorescent microscopy and Ziehl–Neelsen for AFB diagnosis, Intern. J. Tuberc. Lung Dis. 13 (7) (2009) 836–841.
[5] M. Attallah, A.C.A. Malak, H. Ismail, A.H. El-Saggan, M.M. Omran, A.A. Tabll, Rapid and simple detection of a *Mycobacterium tuberculosis* circulating antigen in serum using dot-ELISA for field diagnosis of pulmonary tuberculosis'', 6 Tuberculosis research and treatment, J. Immunoassay Immunochem. 24 (1) (2003) 73–87.
[6] J.M. Dora, G. Geib, R. Chakr, et al, Polymerase chain reaction as a useful and simple tool for rapid diagnosis of tuberculous meningitis in a Brazilian tertiary care hospital, Braz. J. Infect. Dis. 12 (3) (2008) 245–247.
[7] A. Mishra, A. Singhal, D.S. Chauhan, et al, Direct detection and identification of *Mycobacterium tuberculosis* and *Mycobacterium bovis* in bovine samples by a novel nested PCR assay: correlation with conventional techniques, J. Clin. Microbiol. 43 (11) (2005) 5670–5678.
[8] J.S. Pedersen, I. Clarke, J. Mills, Improved detection of mycobacteria species in formalin-fixed tissue sections, Histopathology 59 (5) (2011) 993–1005.
[9] T. Mustafa, H.G. Wiker, S.G.M. Mfinanga, O. Morkve, L. Sviland, Immunohistochemistry using a *Mycobacterium tuberculosis* complex specific antibody for improved diagnosis of tuberculous lymphadenitis, Mod. Pathol. 19 (12) (2006) 1606–1614.
[10] H. D'Avila, C.M. Maya-Monteiro, P.T. Bozza, Lipid bodies in innate immune response to bacterial and parasite infections, Int. Immunopharmacol. 8 (10) (2008) 1308–1315.
[11] S. Logani, D.R. Lucas, J.D. Cheng, H.L. Ioachim, N.V. Adsay, Spindle cell tumors associated with mycobacteria in lymph nodes of HIV-positive patients: 'kaposi sarcoma with mycobacteria' and 'mycobacterial pseudotumor', Am. J. Surg. Pathol. 23 (6) (1999) 656–661.
[12] T. Ulrichs, M. Lefmann, M. Reich, et al, Modified immunohistological staining allows detection of Ziehl-Neelsen-negative *Mycobacterium tuberculosis* organisms and their precise localization in human tissue, J. Pathol. 205 (5) (2005) 633–640.
[13] Smithw'ıck R.W.(1976): Laboratory Manual For Acid-Fast Microscopy: Public Health Service, Center For Disease Control, Bureau of Laboratories.

[14] A.A. Velayati, P. Farnia, M.R. Masjedi, G. Konstantinovich, Morphological modification by tubercle bacilli: no time for denial, J. Infect. Developing Countries 6 (1) (2012) 97–99.
[15] Purohit M. R., Mustafa T., Wiker H. G., Morkve O., and Sviland L. (2007): ''Immunohistochemical diagnosis of abdominal and lymph node tuberculosis by detecting Mycobacterium tuberculosis complex specific antigen MPT64'', Diagnostic Pathology, vol. 2, no. 1, article 36.
[16] K.M. Dobos, E.A. Spotts, F.D. Quinn, C.H. King, Necrosis of lung epithelial cells during infection with *Mycobacterium tuberculosis* is preceded by cell permeation, Infect. Immun. 68 (11) (2000) 6300–6310.
[17] R.W. Light, Tuberculous pleural effusion: pathogenesis and pathophysiological features, Pleural. Dis. 4 (2001), 182-6.
[18] R.W. Light, Y.S. Erozan, W.C. Ball, Cells in pleural fluid: their value in differential diagnosis, Arch. Intern. Med. 132 (1973), 854-60.
[19] A.P. Fishman, Pulmonary Diseases and Disorders, second ed., McGraw Hill, New York, 1988, p. 2143.
[20] B.O. Onadeko, A.A. Abioye, Needle biopsy of the pleura in Nigeria, Br. J. Dis. Chest 73 (1979), 282-4.
[21] B.R. Maldhure, S.P. Bedarkar, H.R. Kulkarni, S.P. Papinwar, Pleural biopsy and adenosine deaminase in pleural fluid for the diagnosis of tubercular pleural effusion, Indian J. Tuberc. 41 (1994), 161-5.
[22] J.R. Tomlinson, S.A. Sahn, Invasive procedures in the diagnosis of pleural diseases, Semin. Respir. Med. 9 (1987), 30-6.